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Involvement of the Purinergic P2X7 Receptor in the Formation of Multinucleated Giant Cells

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Multinucleated giant cells (MGC), a hallmark of chronic inflammatory reactions, remain an enigma of cell biology. There is evidence implicating the purinergic P2X7 receptor in the fusion process leading to MGC. To investigate this, we used HEK 293 cells stably transfected with either 1) the full-length rat P2X7 receptor (P2X7 cells), 2) a rat P2X7 receptor lacking the C-terminal domain (P2X7TC), or 3) a mock vector, and rat alveolar macrophages (MA) expressing the native receptor. P2X7 cells cultured in serum-free medium formed increased numbers of MGC and displayed a higher fusion index compared with mock transfectants. Stimulation of P2X7 pore-forming activity in P2X7 cells by polymyxin B (PMB) further increased significantly the formation of MGC. Conversely, blockers of P2X receptors including oxidized ATP, brilliant blue G, and pyridoxal phosphate-6-azophenyl-2’-4’-disulfonic acid inhibited significantly MGC formation in both unstimulated and PMB-stimulated P2X7-transfected cells. In contrast, cells transfected with the truncated P2X7TC were devoid of pore-forming activity, did not respond to PMB stimulation, and failed to form enhanced numbers of MGC, thus behaving as mock transfectants. As found for P2X7-transfected cells, PMB also potentiated dose-dependently the formation of multinucleated MA by rat alveolar MA. Pretreatment with oxidized ATP abrogated the PMB stimulatory effects. Together, these data demonstrate unequivocally the participation of P2X7 receptor in the process of MGC formation. Our study also provides evidence suggesting that stimulation of the P2X7 receptor pathway in MA may mediate increased formation of MGC during chronic inflammatory reactions. 

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allopurinol and captopril, two agents with therapeutic effect on sarcoidosis, down-regulated the P2X7 receptor and inhibited MGC formation (28). Expression of the P2X7 receptor has also been observed on MA and FBGC during the foreign body reaction to implanted biomaterials (29). The P2X7 receptor was found to be expressed on osteoclasts and their precursors in vitro and on human osteoclasts lining bone surface (30, 31). In addition, fusion of osteoclast precursors to form multinucleated osteoclasts in vitro has been shown to be inhibited by oATP and a P2X7 receptor mAb (32) and, more recently, by down-regulation of P2X7 expression (33). However, the evidence linking P2X7 to MGC formation has been indirect or circumstantial. In addition, recent work has shown that mice lacking the P2X7 receptor were able to form multinucleated osteoclasts in vitro and in vivo (34), thus bringing into question the role of P2X7 in osteoclasts.

Despite numerous observations that FBGC, Langhans’ giant cells, and osteoclasts display distinct cell morphology, and that their respective formation requires a different set of cytokines and receptors, it is noteworthy that the P2X7 receptor has been implicated in the formation of all three types of MGC. It is quite possible that P2X7 is a common molecular step crucial for MGC formation during various types of granulomatous reactions. In this study, we took advantage of a heterologous cell system expressing either the rat P2X7 receptor or a truncated defective receptor, and demonstrate unequivocally the participation of P2X7 receptor activity in the cell-to-cell communication process leading to MGC formation. We also provide evidence that enhancement of the P2X7 function in rat alveolar MA by polymyxin B (PMB) stimulates cell fusion into MGC.

Materials and Methods

Animals and reagents

Lung pathogen-free male Wistar rats weighing 225–250 g were purchased from Harlan World. These animals were shipped behind filter barriers and housed in isolated temperature-controlled quarters under pathogen-free conditions in an animal isolator unit (Johns Scientific). They were given standard laboratory chow and water ad libitum and were used within 2 wk. Approval was obtained from the Animal Care and Use Committee of the University of Ottawa for all procedures. IMDM was purchased from In- damental Technologies. Lab-Tek culture chambers were obtained from Nalge Nunc International. PMB, ATP, oATP, poly-L-lysine, brilliant blue G (BBG), and pyridoxal phosphate-6-azophenyl-2/H11032 have been shown to be inhibitors of MGC formation in vivo (30, 31).

P2X7-dependent pore formation and increases in plasma membrane permeability were measured by monitoring over time the uptake of the fluorescent dye EtBr as described previously (36). Briefly, cells (5 × 10^5/ml) were incubated in a standard saline solution containing 125 mM NaCl, 5 mM KCl, 1 mM MgSO4, 1 mM Na2 HPO4 (pH 7.12), at 37°C. After a 30-min incubation at room temperature, the cDNA precipitate was added to the dish dropwise and gently swirled. The culture medium was changed on the third day, and on the fourth day G418 (0.8 mg/ml) was added to selected transfected clones. Following clone selection, the selective medium contained G418 (0.2 mg/ml) and stable clones were maintained in culture medium containing G418 at the same concentration.

Isolation of rat alveolar MA

MA were recovered from normal rats by bronchoalveolar lavage (BAL) as described previously (10). Briefly, the lungs were lavaged with seven 7-ml aliquots of sterile PBS (pH 7.4) (Wisent), and BAL cells were obtained by centrifugation at 200 × g at 4°C for 5 min. The cells were resuspended in IMDM supplemented with 10% dialysed PBS (Wisent), 0.005% gentamicin (Schering), and 0.8% HEPES, which will henceforth be referred to as MA complete culture medium (MACM). Cells were counted in a hemacytometer chamber, and viability (98–100%) was determined by trypan blue exclusion. Differential analysis of lavage cells made by cytocentrifugation (Thermo Shandon; 2.5 × 10^5 cells) and stained with Wright-Giemsa indicated that the BAL cell population was essentially composed of MA (99%) in normal rats.

Culture and generation of MGC

The generation of MGC from rat alveolar MA was performed using our previously described microculture cell system (10, 11). Briefly, 2 × 10^5 MA were plated in 8-well Lab-Tek tissue culture chambers in 200 μl of MACM and cultured overnight. The cells were then incubated in the presence or absence of PMB at various concentrations as indicated, and for various times (3–5 days) at 37°C in 5% CO2. For the generation of MGC in HEK 293 cell cultures, HEK 293 cells transfected with either the rat P2X7, the rat truncated P2X7 (P2X7TC), or a mock vector, were plated (2 × 10^5) in 2-well Lab-Tek tissue culture chambers coated with poly-L-lysine in 2 ml of DME/F-12 [1:1] containing 10% heat-inactivated FCS. Following overnight incubation, the cells were washed with prewarmed PBS, and cells were incubated in 2 ml of serum-free medium in the presence and absence of PMB at the indicated concentration for various times (24–72 h) at 37°C in 5% CO2. Following incubation of MA and HEK cells, the medium was removed and cells were washed three times with PBS. After drying, the chambers were disassembled, stained with H&E, and mounted with coverslips and Permount (Fisher Scientific) for microscopic evaluation. Ten fields at magnification >330 (or when indicated 20 fields and magnification >165) were imaged on each slide, and data were calculated using the following: f.i. = total number of nuclei in MGC/total number of nuclei counted × 100.

Expression and localization of rat P2X7 receptor

Expression of the P2X7 receptor and its localization were assessed with HEK 293 cells stably expressing the rat P2X7-GFP (rP2X7-GFP). The cells were seeded on 24-mm coverslips in standard saline solution (described in the text) and kept at 37°C. Images of cells transfected with rP2X7-GFP were acquired with a Nikon Eclipse TE 300 inverted microscope (Nikon) equipped with a thermostated chamber (Biophtechs), a 63× immersion objective, and the following filter set: excitation HQ400/ 40, dichroic Q480LP, and emission HQ510LP. For high-speed acquisition and processing of fluorescent images, the microscope was equipped with the following devices: a computer-controlled light shutter, a six-position filter wheel, a piezoelectric z-axis focus device, a back illuminated 1000 × 800 charge-coupled device camera (Princeton Instruments), and a computer with MetaMorph software (Universal Imaging) for image acquisition, two- and three-dimensional visualization and analysis.

Pore-forming activity

P2X7-dependent pore formation and increases in plasma membrane permeability were measured by monitoring over time the uptake of the fluorescent dye EtBr as described previously (36). Briefly, cells (5 × 10^5/ml) were incubated in a standard saline solution containing 125 mM NaCl, 5 mM KCl, 1 mM MgSO4, 1 mM Na2 HPO4, 5.5 mM glucose, 5 mM NaHCO3, 1 mM CaCl2, and 20 mM HEPES (pH 7.6) in a thermostated (37°C) fluorometric cuvette under constant magnetic stirring with EtBr (20 μM). Cells were incubated for 5 min in the presence or absence of PMB (10 μg/ml) and then stimulated with ATP (1 mM). Uptake of the fluorescent dye was monitored for 25 min. At that time, diethion (100 μM) was added to achieve complete permeabilization of cells (100% fluorescence signal). Fluorescence was measured at 360 nm/580 nm excitation/emission wavelengths.
Pore-forming activity was also assessed by determining the percentage of cells that became permeabilized to EtBr, as described previously (37). For this process, HEK 293 cells (4 × 10^4) were incubated in 200 μl of complete medium in 96-well tissue culture plates for 24 h. The medium was then replaced with standard saline solution. EtBr (20 μM) was added, and cells were incubated at 37°C for 5 min in the presence and absence of PMB. Cells were then stimulated with ATP (0.5 mM) for 5 min at 37°C. After centrifugation (200 × g, 1 min), culture supernatants were replaced with fresh saline solution, and fluorescence was analyzed using a Zeiss Axiovert S1100TV inverted microscope equipped with a rhodamine filter and a 32× objective. Images were captured and analyzed with the Northern Eclipse image analysis software. Data are expressed as percentage of cells that become permeabilized to EtBr.

Pharmacological modulation of MGC formation

Cells were cultured in Lab-Tek chambers as described above. For assessment of P2X7 antagonists, cells were pretreated for 2 h at 37°C with αATP (100 μM or 500 μM, as indicated), washed in serum-free medium, and cultured as described above for 48–72 h in the presence and absence of PMB at various concentrations. In some experiments using HEK 293 cells, BBG (5 × 10^-8 M) or PPADS (5 × 10^-7 M) were added 5 min before PMB and cells cultured for 48 h.

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance was determined using one-way ANOVA and Bonferroni test (p < 0.05; Instat; GraphPad).

Results

Enhanced MGC formation in HEK 293 cells transfected with the rat P2X7 receptor

HEK 293 cells transfected with the rat P2X7 receptor and cultured in serum-free medium for intervals up to 72 h undergo fusion into MGC. The presence of MGC with three nuclei was already apparent at 24 h and for up to 72 h. At 48 h, which was the optimal time point chosen for most experiments, cell fusion was observed between MGC and single cells as well as between MGC themselves (Fig. 1, upper left and right panels), indicating that once formed, MGC continue to express at least initially the molecular effector(s) responsible for the fusion process. This is consistent with the notion that MGC are involved in further recruitment and fusion of additional cells leading to large MGC with numerous nuclei as seen during granulomatous inflammatory reactions. Fluorescence microscopy using HEK 293 cells transfected with a rat P2X7 receptor fused with GFP at the C-terminal, further demonstrated the localization of GFP-tagged P2X7 receptor to the cell membrane (Fig. 1, lower right panel). The levels of surface vs intracellular expression can be appreciated from the intensity of GFP-P2X7 fluorescence as depicted on a rainbow scale going from blue (low level) to red (high level) (Fig. 1, lower left panel). In contrast, HEK 293 cells transfected with a control GFP-vector alone (Fig. 1, inset, lower left panel) expressed only cytoplasmic GFP without membrane localization.

Although the presence of MGC was found in cultures of mock-transfected cells, cells expressing the P2X7 receptor had a higher f.i. and formed an increased number of MGC (Fig. 2, A and B). Detailed analysis of the number of nuclei contained within the MGC population of P2X7 cells and mock cells further demonstrated that after 48 h incubation, P2X7-expressing cells display 1.3- and 2.1-fold increase in MGC harboring three and four nuclei, respectively (Fig. 2C).

PMB potentiates both P2X7 pore opening and MGC formation in P2X7-transfected cells

The natural cationic peptide, PMB, has recently been shown to potentiate ATP-induced membrane permeabilization and pore formation, a hallmark of P2X7 activation (36). We next investigated

![FIGURE 1. Phenotypic characteristics of P2X7-deprived MGC. HEK 293 cells transfected with the rat P2X7 receptor were cultured in Lab-Tek chambers for 48 h as described in Materials and Methods. Cells expressing P2X7 undergo fusion into MGC with randomly dispersed nuclei (up to 10) (upper left panel). Some cells display numerous nuclei arranged in a ring-like fashion (upper right panel). Note the fusion between MGC and single cells as well as between MGC themselves. Fluorescence microscopy demonstrates the localization of GFP-tagged P2X7 receptor at the cell membrane of adjacent cells (lower right panel) in contrast to cells transfected with a control GFP-vector alone (inset); the intensity of GFP-P2X7 fluorescence is shown on a rainbow scale going from blue (low level) to red (high level) (lower left panel).]()}
whether activation/promotion of P2X7 would result in further enhancement of MGC formation. To this aim, HEK 293-P2X7 cells and HEK 293-mock cells were incubated in the presence of the impermeant fluorescent dye EtBr (20 μM) and then stimulated with ATP (1 mM) in the presence or absence of PMB. Preincubation with PMB (10 μg/ml) increased significantly the permeabilizing activity of ATP as measured by monitoring the rate of EtBr uptake over a 25-min interval (Fig. 3A). In the presence of ATP, the fluorescent dye could be seen in the nuclei and diffusely throughout the cytoplasm of treated cells upon microscopic observation. Up to 59.9% of P2X7 cells became permeabilized to ATP, and such percentage increased to 79.4% when cells were preincubated for 5 min with PMB (Fig. 3B). Under
The P2X7 receptor C-terminal domain is required for MGC formation

Although the cytosolic C-terminal region of the P2X7 receptor in HEK 293-P2X7 cells has been shown to interact with several proteins (41) and is required for pore-forming activity (23), the site of action of PMB appears to reside within the extracellular domain (36). Therefore, it is quite possible that the bulky extracellular domain of P2X7 might be involved in intercellular communication and MGC formation. To verify this, we studied pore-forming activity and MGC formation in HEK 293 cells transfected with a rat P2X7 receptor lacking the intracellular C-terminal 180 aa (P2X7-TC). We have shown previously that these cells express the rat P2X7 receptor lacking the intracellular C-terminal 180 aa (P2X7-TC) on their plasma membrane as evidenced by their ability to display well-established responses to ATP, including significant [Ca2+]i increase and membrane depolarization (36) that can be potentiated by PMB (36). As expected, HEK 293-P2X7, TC cells lacked pore-forming activity in response to ATP even after pretreatment with PMB, as determined by monitoring the rate of EtBr uptake.

Enhanced MGC formation in P2X7-transfected cells is inhibited by P2X7 blockers

Antagonists at the P2X7 receptor were tested for their ability to interfere with MGC formation in HEK-P2X7 cells. Preincubation with a 5 mM oATP (500 μM), an irreversible blocker of P2X7 function (38), inhibited MGC formation both in unstimulated cells (77% of control) and in cells stimulated with PMB (53–57% of control) (Fig. 5A). Similarly, BBG and PPADS, two antagonists of the rat P2X7 receptor known to block P2X7 pore-forming activity (37, 39, 40), both decreased the number of MGC and the f.i. in unstimulated (71–78 and 72–75% of control, respectively) and PMB-stimulated P2X7 cells (54–63 and 60–68% of control, respectively). In contrast, none of the antagonists inhibited the formation of MGC seen in mock transfectants (Fig. 5B).

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FIGURE 4. PMB potentiates the formation of MGC in P2X7-transfected cells. HEK 293-P2X7 and HEK 293-mock cells were incubated in Lab-Tek chambers for 48 h, as described in Materials and Methods, in the presence and absence of PMB (10 μg/ml and 50 μg/ml). PMB was added at the beginning of the culture. A and B, Enhanced MGC formation was observed in P2X7 compared with mock cells. Incubation with PMB further increased the number of MGC in P2X7 cells but not in mock cells as determined by the percentage of MGC (A) and the f.i. (B). A, Inset. Data are also expressed as percentage (%) of control (P2X7-transfected cells incubated without PMB). Data represent mean ± SEM of at least seven experiments. Significantly different from control at *, p < 0.05 and **, p < 0.01. C, Detailed analysis of the number of nuclei present in unstimulated and PMB (10 μg/ml, P10; 50 μg/ml, P50) stimulated P2X7-transfected cells. Data represent the mean ± SEM of at least four experiments. Significantly different from control at *, p < 0.05, **, p < 0.02, and ***, p < 0.01.
uptake by cells (Fig. 6A) and the number of fluorescent permeabilized cells (Fig. 6B). Similarly, cells transfected with the truncated receptor did not exhibit higher MGC number and f.i. and failed to form increased numbers of MGC in response to PMB (10 and 50 μg/ml), thus behaving as HEK 293-mock cells (Fig. 6C). In contrast, parallel cultures of P2X7 cells displayed higher MGC numbers and f.i. that were further increased following incubation with PMB.

**PMB potentiates MGC formation in rat alveolar MA cultures**

Pure populations of rat resident alveolar MA express a functional P2X7 receptor (37) and represent a useful cell culture system for the generation of MGC following appropriate stimulation (10, 11). We hypothesized that PMB might also modulate positively P2X7 function in rat alveolar MA expressing the native receptor and promote MA multinucleation and MGC formation. To test this theory, MA were incubated with and without PMB at various concentrations in the presence and absence of oATP for 72 h, and their f.i. was monitored. Under our culture conditions, unstimulated MA undergo cell fusion and form multinucleated MA at low levels. Addition of PMB at the beginning of cell culture increased in a dose-dependent manner the formation of MGC with a maximal f.i. of 3.2 obtained at 5 μg/ml PMB compared with 1.4 for unstimulated cells (up to 2.3-fold stimulation) (Fig. 7A). Pretreatment with oATP blocked the stimulatory effects of PMB at all doses tested. Promotion of MGC formation by PMB was maximal at the 3-day time point and decreased thereafter (Fig. 7B). Detailed analysis of
MGC formed in response to PMB showed an increase in the number of multinucleated MA as determined by the percentage of MGC (%) and the f.i. Data represent means ± SEM of three experiments. *, Significantly different from control at p < 0.05. C, MA were incubated in the presence and absence of PMB (10 μg/ml) for 3, 4, and 5 days. Incubation with PMB increased the number of multinucleated MA as determined by the percentage of MGC (%) and the f.i. Data represent means ± SEM of three experiments. *, Significantly different from control at p < 0.01. C, The percentage of multinucleated MA with three or four nuclei was evaluated in unstimulated and PMB (10 μg/ml) stimulated MA pretreated or not with oATP, as described above. Data represent means ± SEM of three experiments. *, Significantly different from control following incubation with PMB at p < 0.05. ***, Significantly different from control following pretreatment with oATP at p < 0.05.

Discussion
In this study, we used an heterologous cell system expressing the P2X<sub>7</sub> receptor to investigate its role in the process of cell-to-cell communication leading to MGC formation. This experimental approach permitted us to single out the participation of the P2X<sub>7</sub> receptor in this process from other P2X receptors, cytokines, and membrane molecules present in cells expressing the native receptor. We present evidence that increased numbers of MGC can be generated from P2X<sub>7</sub>-transfected cells, indicating that P2X<sub>7</sub> receptor expression at the cell membrane can provide sufficient stimulus for induction of such intercellular communication pathways. Our results support earlier work that showed a correlation between P2X<sub>7</sub> expression and giant cell formation (9, 26) and blockade of this process by an inactivating P2X<sub>7</sub> mAb (25). However, expression of the P2X<sub>7</sub> receptor is not a requisite per se for the process of multinucleation, because mock-transfected cells that do not express P2X<sub>7</sub> undergo fusion into MGC, albeit to a lower level. It is likely that other plasma membrane molecules expressed by HEK 293 cells are involved in triggering cell multinucleation. Our results are consistent with previous findings that mice lacking the P2X<sub>7</sub> receptor (P2X<sub>7</sub><sup>−/−</sup>) retain the capacity to form multinucleated osteoclasts (34).

Interestingly, expression of the P2X<sub>7</sub> receptor is associated with a greater ability to form MGC, as evidenced by increased numbers of MGC and higher f.i. in P2X<sub>7</sub>-transfected cells compared with mock cells. Furthermore, we demonstrate that the natural cationic peptide PMB augments P2X<sub>7</sub>-pore-forming activity and enhances MGC formation selectively in P2X<sub>7</sub>-transfected cells but not in mock transfectants. These results confirm previous reports that PMB modulate selectively P2X<sub>7</sub>-receptor function (36) and present the first evidence that PMB can enhance P2X<sub>7</sub>-dependent MGC formation. Under our culture conditions, PMB (10 μg/ml), which is not toxic (36), caused by itself a small but significant increase in the number of P2X<sub>7</sub>-cells permeabilized to EtBr but had no effect on mock cells. Whether this reflects a direct stimulatory effect of PMB on P2X<sub>7</sub>, or is related to our experimental culture conditions is not known and would require further investigation. A direct effect of PMB, even small, on P2X<sub>7</sub> cells would be consistent with our observations of enhanced MGC formation in these cells in the absence of exogenous stimulation with ATP. It should be pointed out, however, that the most striking effect of PMB on P2X<sub>7</sub> pore-forming activity is to potentiate the effect of ATP at the P2X<sub>7</sub> receptor and increase its sensitivity to lower ATP concentration (36). This could be the case during MGC formation because P2X<sub>7</sub>-transfected cells release in the culture medium ATP at levels sufficient to promote activation of P2X<sub>7</sub> in the presence of PMB (42, 43).

Although the process of MGC formation requires 24 to 48 h, it is likely that PMB action is rapid because the peptide would be rapidly metabolized under normal culture conditions. This implies that the PMB effect could be related to brief activation of P2X<sub>7</sub>. In support of this, potentiation of ATP-induced cell permeabilization was not observed in P2X<sub>7</sub>-transfected cells incubated overnight with PMB (our unpublished results). MGC formation is a process that requires concerted cytoskeletal reorganization and regulated plasma membrane disruption. It is tempting to speculate that MGC formation may be associated with the reversible pseudoapoptotic signaling pathway induced by brief activation of P2X<sub>7</sub> receptors (44). In support of this, experimental evidence has demonstrated that intercellular fusion leading to multinucleation may occur through mechanisms that are independent of apoptosis (45, 46). In contrast, overexpression of P2X<sub>7</sub> has been associated with cell death (24), and PMB has been shown to augment P2X<sub>7</sub>-dependent cytotoxicity (36). Therefore, the possibility remains that MGC formation results from the fusion of cells programmed for death. Further experimentation is currently underway to address these issues.
One question that has been left unanswered with regard to the P2X7 receptor is whether a functional receptor is needed for membrane fusion leading to MGC. It has been proposed that in partner cells expressing P2X7, activation of the receptor could generate a “fusion pore” that would bridge the cytoplasm of the adjacent cells and drive the eventual fusion (25). In this study, we demonstrate that cells transfected with a P2X7 receptor truncated at the C-terminal domain and which fail to induce pore formation in response to ATP, also lose the ability to form increased numbers of MGC, even in the presence of PMB thought to act at the extracellular domain (36). Our data provide direct evidence that MGC formation requires an integral C-terminal domain and a functional receptor. In accordance with this result, previous work has shown that the formation of multinucleated osteoclasts was lost in cells that were resistant to ATP but was recovered in cells that regained P2X7 pore-forming activity (33), suggesting that P2X7 might be involved in the mechanics of cell fusion or, alternatively, in a signaling pathway proximal to this event. However, it is difficult to link MGC formation to the pore-forming activity in our study because the cytosolic C-terminal region of the P2X7 receptor has been shown to be important for many P2X7-related functions beside pore formation including membrane blebbing, and apoptosis. Little is known about how the functional domains of the C-terminal region contribute to the numerous properties ascribed to P2X7. As mentioned earlier, the process of multinucleation requires cytoskeletal rearrangements and regulated membrane disruption, and the C-terminal tail of P2X7 interacts with several proteins that could potentially drive such a process including actin, actinin, and β2 integrin (41).

It is noteworthy that the observations made with an heterologous cell system expressing no other P2X receptors than P2X7, can be translated in rat alveolar MA expressing the native receptor. Rat alveolar MA have the potential to form MGC in vivo (47) and in vitro (10, 11, 18) and exhibit a functional P2X7 receptor that, upon stimulation with ATP, mediates pore formation and activates the proinflammatory IL-1β–IL-6 cytokine cascade (37). We provide further evidence that activation of P2X7 by PMB in these cells increased their ability to form MGC. As discussed for P2X7-transfected cells, extracellular ATP released by alveolar MA under our culture conditions, even at low levels, may act in concert with PMB to promote P2X7 receptor activation and MGC formation. In accordance with earlier findings (36), our data point to PMB as a selective tool to increase P2X7-mediated function in rat alveolar MA.

Altogether, our observations provide direct evidence that expression and activation of the P2X7 receptor is associated with increased cell-to-cell fusion and enhanced formation of MGC. Thus, P2X7-mediated MGC may represent a separate, amplification pathway aimed at augmenting the process of MA multinucleation during host response to danger signals and chronic granulomatous reactions. In support of such an assumption, activation of P2X7 in MA has been linked to several responses relevant to inflammation including NF-κB, cytokine and NO release, superoxide production, and cytotoxicity (48). It is likely that intercellular communication is crucial in coordinating MA responses during inflammatory reactions, and our results point to the P2X7 receptor as an efficient system for the promotion and maintenance of intercellular communication during such reactions. This is directly relevant to recent views that chronic inflammation is the product of pathologic cell-cell interactions, occurring within a unique and defined biological compartment such as the lung, gut, and synovium. Understanding the mechanisms underlying the participation of the P2X7 receptor in cell-to-cell communication, may provide a basis for the development of strategies aimed at interfering with pathologic MA-MA interactions in diseases.

Disclosures

The authors have no financial conflict of interest.

References


